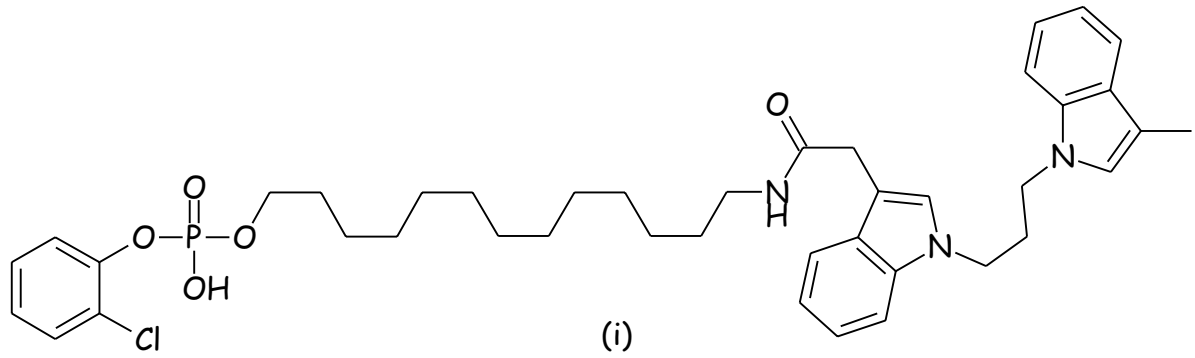
Current literature highlights Vol.4 No.8 April 2002

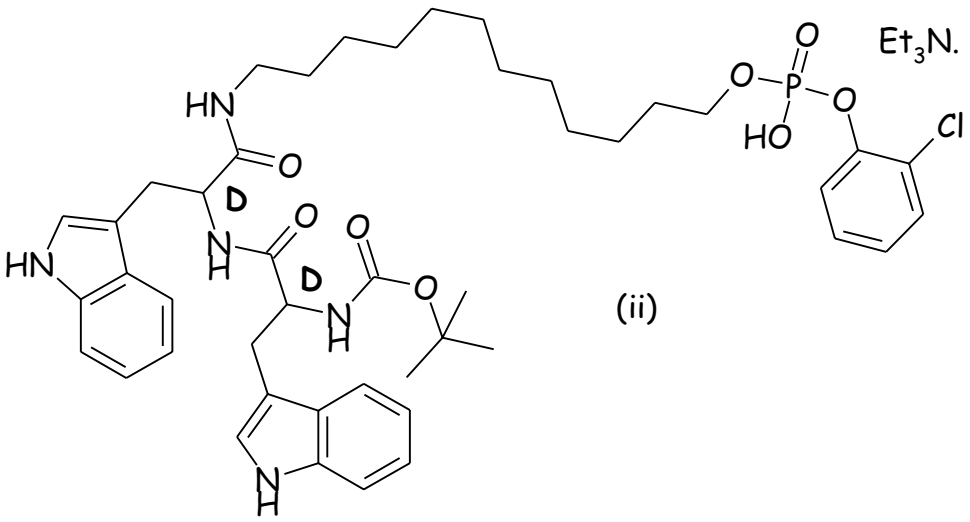
Telomerase Inhibitors

Telomerase is the enzyme responsible for maintaining telomere length and it has activity not observed in normal somatic cells. In contrast, high expression of telomerase is observed in around 85-90% of human tumour cells and therefore telomerase is regarded as a specific target for development of cancer chemotherapeutic agents. There are several types of inhibitor known. For example antisense oligodeoxynucleotides and related compounds which exhibit potent inhibition of telomerase in the picomolar range. In spite of this research there have been no clinical trials of inhibitors to date, and discovery of novel inhibitors will contribute to evaluation of telomerase inhibitors for cancer chemotherapy. Recent developments have highlighted new telomerase inhibitors based on the bisindole unit (i) (S. Sasaki *et. al*., *Bioorg. Med. Chem. Lett*., 11, (2001), 583).



(i)

These new inhibitors are constructed of a simple assembly of a phosphate with a hydrophobic group, a bisindole unit, and a long alkyl spacer between them. Such a simple structural feature of these inhibitors has led to the search for more potent inhibitors (Solid-phase synthesis of a library constructed of aromatic phosphate, long alkyl chains and tryptophan components, and identification of potent dipeptide telomerase inhibitors, S. Sasaki *et. al*., *Bioorg. Med. Chem. Lett*., 11, (2001), 2581-2584). A small library of 42 single compounds was synthesised on Merrifield solid phase resin, and upon cleavage, evaluation of the library compounds' ability to inhibit telomerase, testing in a quantitative stretch PCR assay with the use of telomerase extracted from HCT116 (American Type Culture Collection), revealed several potent compounds. One of the most potent isolated was (ii) which possessed an IC_{50} of 300 nM. As other stereoisomers were either inactive or much less active, the dependency on the stereochemistry has suggested that there should be some stereospecific demand in the enzyme binding sites for this series of inhibitors. This information, along with other SAR generated in the library, may contribute to the future development of potent telomerase inhibitors.



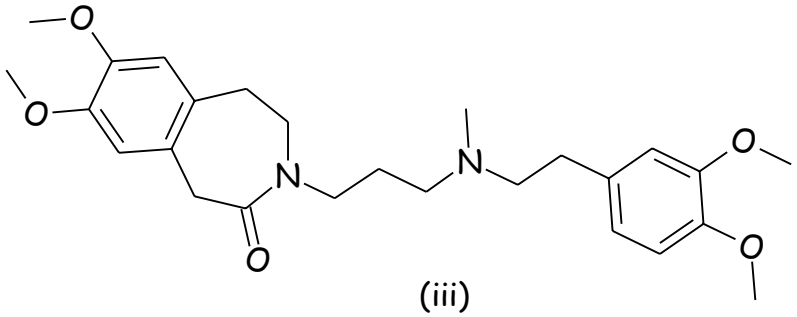
Library design biased against mutagenic compounds

The use of high-throughput screening has represented a major advance in the process of lead discovery. Whenever the paradigm is applicable, the use of robotics and large chemical library screening has yielded uneven results that are quite dependent on the families of targets, but could result in the identification of multiple hits for an assay. In many cases, the hits found had to be subsequently discarded because of their toxicological profile or poor bioavailability. If compounds are to be used as drugs, certain biases are necessary to limit the range of properties of the compounds used for building up a library to those relevant to pharmaceuticals. Toxicology, such as mutagenicity, is important in defining the viability of a chemical as a candidate for drug development. A study on comparative analysis of simple physical characteristics of compounds that have been reported to be mutagens, or non-mutagenic, has been carried out analysing for differences that can lead to the development of knowledge-based biases in libraries designed for mass screening (Towards the design of chemical libraries for mass screening biased against mutagenic compounds, H.O. Villar, *J. Med. Chem*., 44, (2001), 2793-2804).

For each of four Salmonella strains, TA-98, TA-100, TA-1535, and TA-1537, an analysis of the statistical significance of the deviance of the averages for a number of global properties was carried out. The properties studied included parameters such as topological indices and bit strings representing the presence or absence of certain chemical moieties. The results suggest that mutagens display a larger number of hydrogen bond acceptor centres for most strains. Moreover, the use of bit strings points to the importance of certain molecular fragments, such as a nitro group, for the outcome of a mutagenicity study. Development of multivariate models based on global molecular properties or bit strings point to a small advantage of the latter for the prediction of mutagenicity. The purpose of this work was the analysis of biases in the structure and molecular properties of the compounds that are found to be mutagens. The properties most commonly used for library design, bit strings and global properties, show the ability to discriminate among Ames positive and negative compounds for each Salmonella strain selected. These properties can then be used for diversity analysis but also to provide constraints to further tailor libraries towards compounds more likely to be developed into drugs.

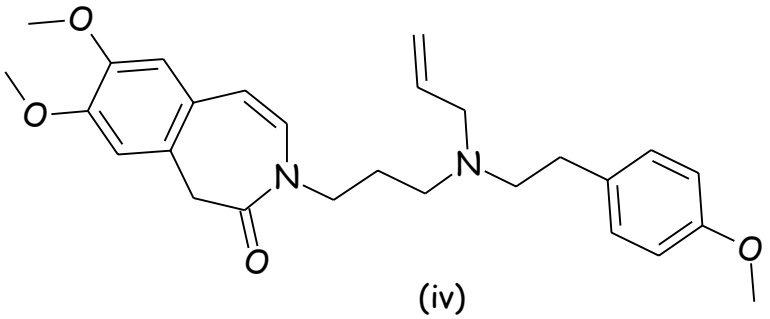
I_f channel blockers

Channel-mediated currents consisting of small background currents such as I_{K1}, I_B and I_f, and large voltage-gated currents such as I_{ca} and I_K play an important role in the generation of spontaneous diastolic depolarisation and action potential of cardiac pacemaking cells. They can contribute to changes in spontaneous pacemaker activity, affecting slow diastolic depolarisation and hence threshold potential for action potential generation and resulting potential of pacemaker cells. Selective reduction in heart rate with no important changes in contractility and wall tension may have several advantages in the treatment of ischaemic heart diseases. Zatebradine (**iii**) is a representative compound of the therapeutic class of sino-atrial node modulators which have been shown to inhibit the hyperpolarisation activated current I_f. Selective blockade of the I_f current causes a slowing down of the spontaneous rate of firing of the pacemaker cells without resulting in abolition of pacemaker activity.



(iii)

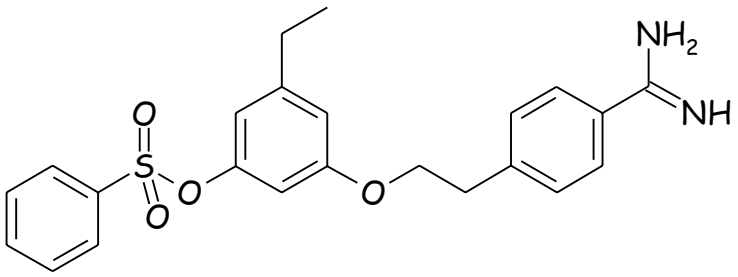
The first solid phase synthesis of zatebradine and its analogues has been reported (Parallel solid-phase synthesis of zatebradine analogues as potential I_f channel blockers, Booth, S. *et. al*., *Bioorg. Med. Chem. Lett*., 11, (2001), 2351-2354). A small library of 21 single analogues was prepared on REM solid phase resin. Upon cleavage, evaluation of the library compounds ability to reduce the spontaneous beating of isolated guinea-pig atria in a concentration-dependent manner was undertaken. One of the most potent isolated was (**iv**) which showed a maximum reduction of beating of 80% at 3 μM compared to a reduction of 40% at 3 μM with Zatebradine (**iii**). This library has resulted in the discovery of a series of compounds with increased ability to reduce the spontaneous beating of isolated guinea-pig atria. Further studies are warranted to elucidate these compounds' putative actions on calcium channels.



(iv)

Thrombin inhibitors

Thrombosis is the result of improper regulation of the hemostasis mechanisms, leading to the formation of intravascular clots which may cause tissue damage or cell death due to inadequate blood flow. This cardiovascular disorder can lead to deep vein thrombosis, myocardial infarction and stroke. Much of the current drug research is focused on finding an antithrombotic drug that is safe and effective and can be administered orally. One prominent target for such a drug is the enzyme thrombin (factor IIa). Thrombin plays a central role in the blood coagulation process as the final key enzyme in the cascade involved. It converts soluble fibrinogen to fibrin, which forms the fibrillar matrix of the blood clot. Activation of thrombin initiates several other mechanisms including both positive and negative feedback of thrombin generation, together with a variety of cellular effects such as platelet aggregation and tissue remodelling. A low molecular weight substance that can inhibit thrombin, and thus its actions, would be a potentially powerful antithrombotic drug. Finding an inhibitor that possesses both selectivity and suitable pharmacokinetics has been difficult to identify, prompting research in this area to continue (Statistical molecular design, parallel synthesis, and biological evaluation of a library of thrombin inhibitors, Linusson, A. *et. al.*, *J. Med. Chem.*, 44, (2001), 3424-3439). A small library of 18 analogues was prepared in solution. The compounds were analysed with respect to their inhibition (pIC_{50}) of thrombin, their membrane permeability, estimated by migration behaviour in micellar media, their pKa, and their specificity with respect to inhibition (K_i) of trypsin. One of the most potent compounds isolated was (**v**) which possessed a pIC_{50} for thrombin of 7.6.



(v)

This work has provided a series of compounds worthy of further investigation in the search for a low molecular weight substance capable of inhibiting thrombin.